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TITLE: Low Melatonin Production During Adulthood – Phase 2: Association with Levels of Hydroxyl Radical Scavenging and DNA Damage

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14. ABSTRACT

The primary purpose of the study was to develop cross-sectional evidence concerning whether or not lower melatonin production levels are associated with increased oxidative DNA guanine damage. Because the results of this study are supportive, confirmatory studies are warranted, followed by prospective chemoprevention studies of melatonin supplementation. Adjuvant cancer treatment studies have not identified any serious melatonin toxicities.

High-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI MS/MS) was used to simultaneously quantitate urinary levels of 8-hydrodeoxyguanosine (8oxodG), 8-hydroxyguanosine (8oxoGuo), and 8-hydroxyguanine (8oxoGua). Overnight creatinine-adjusted urinary 6-sulphatoxymelatonin (aMT6s) production was assayed in an earlier study. The complete overnight urine samples were properly processed and stored. Fifty-five (55) mother-daughter(s)-father triples of urine samples were available. Fifty-one (51) mothers and 67 daughters were actually assayed. Among the mothers, total overnight 8oxodG was inversely significantly ($p<0.05$) associated with higher levels of aMT6s. Among the daughters, there were no associations with 8oxodG, 8oxoGua, or 8oxoGuo.

15. SUBJECT TERMS
DNA damage, urinary metabolites, urinary melatonin metabolites

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INTRODUCTION

The study is designed to develop preliminary data on whether oxidative DNA damage is increased in women with lower levels of melatonin production. The study uses the complete overnight urine samples collected under Phase 1 (funded by a DOD Concept Grant), with appropriate IRB and DOD approvals. The consent document allowed use of the specimens for the Phase 2 purpose. Specimens and data are identifiable only by code numbers, no longer linked to personal identifiers.

Breast cancer causes a reduction in melatonin production, making post-cancer measurements inappropriate for case-control studies. The objective of Phase 1 was to use a daughter's and her father's current melatonin production levels to estimate the mother's pre-cancer melatonin production level, making breast cancer case-control studies of low melatonin production appropriate. Cumulative overnight urinary 6-sulphatoxymelatonin (aMT6s) production, adjusted for urinary creatinine, was the measure of melatonin production.

Oxidative DNA damage can lead to malignant transformations and is considered an important factor in the development of breast cancer. Estradiol is a major contributor to the production of $HO^{\bullet}$, particularly in breast epithelial cells. $HO^{\bullet}$ has been shown to cause oxidative DNA damage, particularly to guanine. Melatonin appears to be a powerful scavenger of $HO^{\bullet}$. When guanine is oxidized, 8-hydrodeoxyguanosine (8-oxodG), 8-hydroxyguanosine (8-oxoGuo), and 8-hydroxyguanine (8-oxoGua) are excreted in urine. Several studies have found that melatonin inhibits mammary tumor development in animals prone to such tumors or exposed to a carcinogen. In animal studies, melatonin has demonstrated protective capabilities against natural oxidative DNA damage, as indicated by 8-oxodG levels.

BODY

The study had 3 primary tasks:

Task 1: To complete all preliminary work.
Task 2: To conduct the actual laboratory assays.
Task 3: To analyze the data and write the manuscript(s).

Task 1 has been completed, except the inclusion of 3-OHM in the assays proved not to be possible to accomplish in this study for technical reasons.

Task 2 has been completed. High-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI MS/MS) has been used to simultaneously quantitate urinary levels of 8-oxodG, 8-oxoGuo, and 8-oxoGua. This assay appears superior to both the comet and the HPLC-ECD assays (Weimann et al., 2002). Assays to estimate melatonin production were already performed in Phase 1.

8-oxodG, 8-oxoGuo and 8-oxoGua analyses have been completed on 118 women in the original sample, with 67 being daughters and 51 mothers.

The data have been edited and combined with the Phase 1 melatonin metabolite and epidemiologic data.

The analyses for Task 3 have been completed. Manuscripts for publication are scheduled to be completed by the end of December 2005.

KEY ACCOMPLISHMENTS

The study has been completed, except for the writing up of the results for publication. We anticipate that this will be accomplished by the end of 2005. Among the mothers (aged 48+), higher creatinine-adjusted total overnight urinary aMT6s (highly correlated with total overnight melatonin production, see Cook et al., 2000, and Graham et al., 1998) was statistically significantly associated with lower levels of total 8oxodG, a primary urinary metabolite associated with oxidative guanine base DNA damage substantially caused by hydroxyl radicals. Among the daughters of these mothers, there was no relationship between creatinine-adjusted total overnight urinary aMT6s and any of the 3 urinary metabolites of oxidative guanine base DNA damage.

REPORTABLE OUTCOMES

Assays were able to be run for 51 of 55 of the mothers and 63 daughters. Table 1 provides the descriptive statistics for self-reported age, self-reported weight, total overnight creatinine-adjusted aMT6s, and total overnight 8oxoGua, 8oxoGuo, and 8oxodG.

Mothers were, of course, older. Weight was comparable between mothers and daughters. Urinary aMT6s was lower for mothers, as was expected, because generally melatonin production decreases with age.

TABLE 1: DESCRIPTIVE STATISTICS

1. **Age (years)**

	Mean	S.D.	25-75 Percentile	Range
MOTHERS	59.6	10.2	50.2 – 68.4	43.5 – 80.7
OLDEST DAUGHTERS	32.9	9.8	24.3 – 38.4	18.6 – 51.6
ALL DAUGHTERS	32.7	9.6	24.3 – 38.3	18.6 – 51.6

2. **Weight (lbs)**

	Mean	S.D.	25-75 Percentile	Range
MOTHERS	152.1	25.9	131 – 165	107 - 230
OLDEST DAUGHTERS	150.7	42.1	124 – 160	105 - 358
ALL DAUGHTERS	155.6	42.6	130 – 174	105 - 358

3. **Creatinine-Adjusted Total Overnight Urinary Melatonin Metabolite: aMT6s (ng/ml creatinine)**

	Mean	S.D.	25-75 Percentile	Range
MOTHERS	55.5	30.0	33.3 – 80.3	4.0 – 123.2
OLDEST DAUGHTERS	79.6	48.3	45.1 – 100.1	6.1 – 240.8
ALL DAUGHTERS	76.0	47.6	43.7 – 100.1	6.1 – 240.8

4. **TOTAL Overnight 8oxoGua (nmol)**

	Mean	S.D.	25-75 Percentile	Range
MOTHERS	33.9	34.6	14.2 – 36.7	4.3 – 173.8
OLDEST DAUGHTERS	39.7	46.8	15.5 – 42.5	4.8 – 267.3
ALL DAUGHTERS	36.6	41.6	15.7 – 38.4	3.7 – 267.3

5. **TOTAL overnight 8oxoGuo (nmol)**

	Mean	S.D.	25-75 Percentile	Range
MOTHERS	6.3	3.2	3.6 – 8.3	1.3 – 18.6
OLDEST DAUGHTERS	6.6	5.2	3.9 – 7.2	1.7 – 34.1
ALL DAUGHTERS	6.2	4.6	3.7 – 7.4	0.9 – 34.1

6. **TOTAL overnight 8oxodG (nmol)**

	Mean	S.D.	25-75 Percentile	Range
MOTHERS	4.7	2.1	2.9 – 6.2	1.1 – 9.3
OLDEST DAUGHTERS	5.9	4.6	3.6 – 5.9	1.5 – 26.7
ALL DAUGHTERS	5.4	4.2	3.5 – 6.2	0.6 – 26.7

Regressions were calculated with aMT6s as the independent variable and each of the urinary metabolites 8oxoGua, 8oxoGuo, and 8oxodG as the dependent variables. Other independent variables allowed in the regression equations were age and weight, because they have been previously found to be associated with DNA damage. The regressions using mothers and daughters together took into consideration the correlation between the mothers and the daughters. All analyses were conducted using SAS.

We have the following results, using $p \leq 0.05$ for statistical significance and $0.05 < p \leq 0.10$ as marginally significant.

a. Mothers and All Daughters

- ✓ **Total overnight 8oxodG marginally decreases with increased total overnight urinary creatinine-adjusted aMT6s.**

b. Mothers + Oldest Daughters

- ✓ **Total overnight 8oxodG significantly decreases with increased total overnight creatinine-adjusted urinary aMT6s./**
- ✓ Total overnight 8oxodG marginally decreases with weight.

c. Mothers Only

- ✓ **Total overnight 8oxodG significantly decreases with increased total creatinine-adjusted urinary aMT6s.**
- ✓ Total overnight 8oxoGua significantly decreases with age.
- ✓ Total overnight 8oxoGuo significantly increases with weight.

d. Oldest Daughters Only

- ✓ Total overnight 8oxoGua marginally increases with age.

CONCLUSIONS

The data indicate that older women appear to have lower levels of guanine base DNA damage, as measured by total overnight 8oxodG (the most commonly used urinary biomarker for guanine base DNA damage), if they have higher levels of melatonin production. On the other hand, this inverse relationship does not appear to be operating among younger women. This study is the first one to investigate whether increased melatonin production among women at the primary age range for breast cancer is related to lower levels of DNA damage – at least guanine base DNA damage.

It remains to be determined whether this relationship holds for the breast itself, in addition to other organs.

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PERSONNEL RECEIVING SALARY

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APPENDICES

None.